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A new method to force-feed and rear adult newts on board a space station

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Summary

We developed a force-feed method for the adults of the newt, *Pleurodeles waltl* (urodele amphibian) under microgravity conditions on board the Mir space station, because the animals cannot take food themselves under such conditions. A cosmonaut introduced a catheter through the mouth into the stomach. The catheter was connected to a syringe containing food and allowed him to dose the quantity of injected food. The selected food was a complete diet for dogs and cats called "Prescription diet Canine/Feline a/d" made in USA and produced by the Hill's Pet Nutrition SNC (Sofia-Antipolis, France). It was diluted, sterilized and conserved on board the Mir station at ambient temperature (20-30°C). The protocol of force-feeding was successfully performed on board the space station during five months. After the space mission, two females were in good health status. They laid fertilized eggs in flight and again in the ground laboratory after the landing. According to the results obtained with *Pleurodeles* females, such food could be used in future long space flights.

Introduction

We are working on amphibian development under microgravity conditions. During the 1993-1998 period, we performed several experiments during short space flights using *Pleurodeles waltl* embryos or adults. Effects of microgravity on fertilization, embryonic development and post-flight development were studied *(Bautz et al., 1996; Dournon et al., 1997; Husson et al., 1997; Aimar et al., 2000; Dournon et al., 2001; Gualandris-Parisot et al., 2002).* In 1999, the effects of a long space flight, principally on reproduction, inner ear development and immunoglobulin expression, were studied in the experiment called "Genesis" using *Pleurodeles* adults. Concerning the inner ear, the goal was to analyze the aspect and structure of otoconia in developing embryos or larvae and adults on Earth *(Oukda et al., 1999a,b)* and in weightlessness *(Oukda et al., 2001).* To have living embryos and larvae on board the Mir space station, preinseminated females received a hormonal injection to induce spawning *(Aimar et al., 2000; Dournon et al., 2001).* Consequently, it was necessary to rear and feed both larvae and adults in microgravity conditions using adapted techniques. For larvae, the rearing and feeding techniques used during 21 days on board Mir for the experiment Genesis were previously described *(Durand et al., 2000).* In this paper, we report the techniques first used to rear and to feed amphibian adults on board a space station. A French cosmonaut, Jean-Pierre Haigneré, was in charge of the scientific experiments and animals, and successfully practised rearing and feeding them during five months. At the end of the space mission, two surviving females were in good health. They laid fertilized eggs two times during the experiment Genesis, one time during the space flight and the other time in the laboratory after landing.

Materials and Methods

Rearing in standard conditions in laboratory *Pleurodeles waltl* (urodele amphibian) derived from laboratory rearing were treated in accordance with National Legislation and The Council Directive of the European Communities on the Protection of Animals Used for Experimental and Other Scientific Purposes 86/609/EEC. The adults used were three-year old. In standard conditions, *Pleurodeles* adults are reared in large aquariums or plastic basins in tap water at 13-22°C according to the seasons and submitted to the daylight variations *(Dournon et al., 2001)*. Carnivorous, they are fed two times a week with ground meat and *Chiromona plumosus* larvae put into the aquarium. A few hours after each feeding, the animals are placed in clean water. The adults can fast during 4-5 weeks.

Strategy used for the long space flight

The last French-Russian space mission, called "Perseus" occurred in 1999, between February 20 and August 28. On April 2, 1999, the materiel for the Genesis experiment was launched on board the automatic vessel Progress M-41 from the Baïkonur space base toward the Mir space station. It consisted of five 2.8-litre reservoirs containing filtered (0.22 µ) modified physiological rearing medium of Steinberg *(Dournon et al., 2001)*, fourteen syringes containing food for animals and eight *Pleurodeles* adults, four males and four females. After a two-day trip, they were received by the crewmembers. All this material was transported inside a temperature-controlled unit named CTA "Container de Transport Aller" (Fig. 1a) developed by the French space agency (CNES). On board Mir, a space instrument called "Fertile" (Fig. 1b) also developed by the CNES was used to rear the animals *(Husson et al., 2001)*. This space instrument was previously brought to the Mir space station on May 5 1996 to be used three times. Two times for the experiment FERTILE performed in August 1996 and February 1998 *(Aimar et al., 2000; Dournon et al., 2001; Gualandris-Parisot et al., 2002)* and finally for this Genesis experiment. For landing, the living adult females and fixed biological samples were put in a passive container equipped with expansion moss covered with resin and "Nomex" material to limit variations of temperature in absence of electrical supply in the vessel Soyuz. Its design was adapted to the small volume available in the Soyuz. The return trip to Earth lasted about 6 hours.

Synchronous ground experiment

During the space mission, a ground control experiment was performed using an analogous CTA and the "Fertile" instrument. The animals were reared and fed according to the same protocol, techniques and instruments as on board Mir. The ground control experiment was two days delayed compared with the space experiment.

Results

Hardware used to rear Pleurodeles adults on board Mir

In the CTA, three "boxes of transport" (Fig. 1c) were used for the transportation of animals toward the space station, and then used on board to rear adult animals during five months. The CTA was set at 18 ± 2°C and an air pump ventilated (0.2 litre/min) the three boxes. Each was 192 mm in length, 152 mm in width and 47 mm in height with an inner volume of 1 litre. The animals were maintained without water, but a damp towel that covered the two largest internal surfaces, moisturized their skin. The cloth lining was saturated with filtered (0.22 µm) and sterilized physiological rearing medium. At the beginning of the space mission, the four males, smaller than the four females, were grouped in one box and the females were 2 per box. On board Mir, three "egg-laying boxes" (Fig. 1d) were located inside the "Fertile" instrument. The egg-laying boxes were 226 mm in length, 164 mm in width and 76 mm in height. Two polycarbonate portholes allowed visual monitoring of the interior of the box. The internal volume was one litre and each box was ventilated by a system of air circulation (0.2 litre/min). The internal surface was covered with a water-absorbent cloth covering a sponge allowing permanent hydration of the skin of the adults or the jelly coat of eggs. A specific system of water distribution and absorption was designed to control liquid before and after each opening of the box, to prevent water escaping opening of the box, to prevent water escaping into the space station

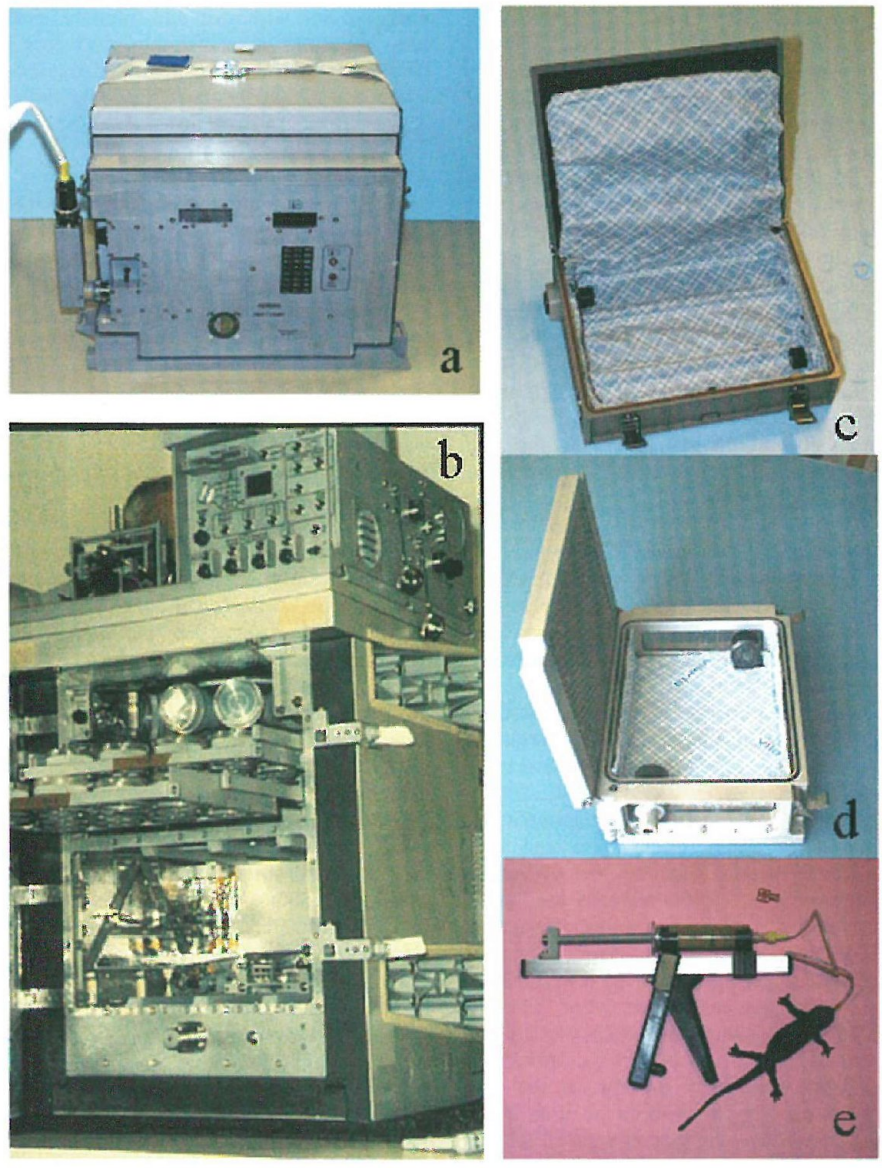
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Fig. 1a - CTA used to transport the living materials toward the space station.
b - The instrument Fertile located on board the MIR space station.
c - Box of transport located in the C.T.A. and used for transportation of adults toward the MIR space station.
c - Egg-laying box located in the instrument Fertile and used to rear the adult females.
e - Syringe with diluted a/d food on a dispenser and connected to a catheter. The pleurodele presented is a plastic model.

and to avoid the drowning of the animals when the boxes were shut. An inflatable bag compressed a sponge against a metallic girdle located between the sponge and the lining. When the bag was inflated, the water was freed inside the box and when it was deflated the sponge absorbed the water. At the beginning of the space mission, one of these boxes was used to rear two females. Later, these boxes allowed spawning by the two surviving females in good conditions.

Technique of rearing on board Mir
Every other day after each feeding, the cosmonaut cleaned the lining and removed scraps using an absorbent paper tissue (Fig. 2a). After three feedings, the lining was changed and moistened. Each time, before closing the box, the cosmonaut moisturized the skin of the animals with the physiological medium using a 100 ml syringe. Each day, he controlled temperature, ventilation and humidity inside the CTA and the "Fertile" instrument.

Technique of nutrition on board Mir
The method routinely used on earth to feed Pleurodeles adults could not be adapted to the microgravity conditions, because the animals could not themselves take food in the absence of free water in the containers used as rearing boxes. Consequently, a force-feed method was developed. This method had the advantage that the cosmonaut controlled the quantity of food distributed per animal. Through the mouth of the animal, he introduced into the stomach a catheter connected to a 20 ml syringe containing food. The catheter was 2.1 mm in diameter and 420 mm in length (Nutrisafe, ref. 361 08, Vygon, Brussels, Belgium). The extremity was rounded and two openings were located laterally. To prevent stomach damage, a colored line traced on the catheter indicated the useful length. For the force-feeding procedure, the cosmonaut had the animal in one hand with the thumb on the animal throat. With small backward movements of the thumb, he opened the mouth of the *Pleurodeles* adult. Using the other hand, the operator laterally introduced the catheter into the mouth, and then dosed the animal at the using a 20 ml syringe with a dispenser (Figs. 1e and 2b).

Choice and characteristics of the selected food.
The food must fulfill several requirements. It must maintain the animals in a good physiological status, particularly for reproduction during the flight. It

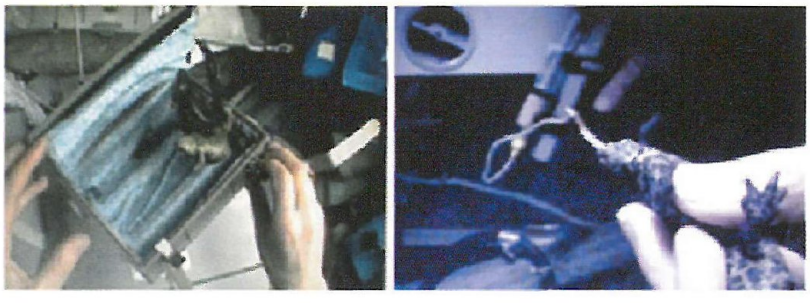

Fig. 2a - On board Mir, the French cosmonaut opening a box of transport with two females. Inside the box, a hydrated cloth.
b - On board Mir, the French cosmonaut force-feeding an animal.

must be sufficiently fluid to move in the catheter and not too fluid to prevent regurgitation. It must be sterilized without alteration of nutritive qualities and conserved six months on board Mir at ambient temperature (20-30°C). The selected food was a complete diet for dogs and cats called "Prescription diet Canine/Feline a/d" made in USA and produced by the Hill's Pet Nutrition SNC (Sofia-Antipolis, France). It was used diluted to obtain the optimal fluidity. With other foods tested the animals lost weight too quickly and could not reproduce.

Ingredients in the food.
The ingredients indicated in the food were: liver, chicken, cornstarch, casein, fish oil, calcium carbonate, sodium tripolyphosphate, plant gum, potassium chloride, choline chloride, taurine, magnesium oxide, zinc oxide, iron sulfate, cupric sulfate, manganese oxide, sodium selenite, calcium iodate, provitamin D, vitamin E, thiamine, niacin, calcium pantothenate, vitamin B6, vitamin B2, folic acid, vitamin H and vitamin B12.

Preparation and rations.
The food was adapted to *Pleurodeles*. The food was diluted with distilled water (4 vol. of food for 1 vol. of water), mixed, and then degassed using a vacuum pump. 20 ml of the homogenous preparation was introduced into 20 ml syringes using the piston. The syringes were then plugged and sterilized.

Sterilization.
IBA Mediris Society (Fleurus, Belgium) performed sterilization of the food loaded syringes using a 25,000 Gray dose with an industrial cobalt-60 gamma irradiator. A total of 114 syringes were prepared for ground control tests and the space flight experiment.

Feeding control tests previously performed on ground
Firstly, to prepare the space experiment, *Pleurodeles* adults were force fed in the ground laboratory during two months with a fresh mixed preparation and the results were positive. The animals did not lose weight too quickly and could reproduce after the force-feeding treatment. Secondly, male and female adults were tested with the sterilized food. They were fed with 1 ml of mixed preparation. When greater quantities were applied, the animals regurgitated the food. They were reared in 5 mm water depth, at 18 ± 2°C and maintained in darkness as in the space station conditions. Two batches of 9 and 12 animals were force-fed during 2 and 6 months, respectively (Tables 1 and 2). In the first batch, the animals were force-fed two times a week during two months. The females (N = 5) and the males (N = 4) lost on average 1.8 g of weight (about -6 %) and 0.8 g of weight (about -3 %), respectively. In the second batch, the animals were force-fed two times a week during two months, and then three times a week during four months. During the first two months, the females (N = 8) and the males (N = 4) lost on average 2.7 g of weight (-7 %) and 1.8 g of weight (-8 %), respectively. At the end of the 6-month force-feeding, the females (N = 8) and the males (N = 2) lost on average 7.9 g of weight (-21 %) and 4.8 g of weight (-20 %), respectively.
In the second batch, the weight loss per month was less during the 3x per week force-feeding periods than during the 2x per week force-feeding periods. More frequent feeding was not possible in the space station because the time the crew had for this experiment was strictly limited.

Table 1. Weight of animals force-fed during 2 months on ground

	Number	Weight at the beginning of force-feeding (g)	Weight after 2 months of force-feeding two times a week (g)	
Females	5	29.6 ± 1.3	27.8 ± 1.4	n.s.
Males	4	26.4 ± 1.1	25.6 ± 1.7	n.s.

n.s. = difference not significant

Table 2. Weight of animals force-fed during 6 months on ground

	Number	Weight at the beginning of force-feeding (g)	Weight after 2 months of force-feeding two times a week (g)	Weight after 4 months of force-feeding three times a week (g)	
Females	8	37.5 ± 1.3	34.8 ± 1.4	29.6 ± 2.1	n.s. then s.
Males	4	23.6 ± 1.1	21.8 ± 1.7	18.8 ± 0.6	n.s. then s.

Consequently, for the in-flight and synchronous ground control experiments, the performed protocol was to feed all the animals with 1 ml of diluted preparation three times a week.

Feeding during five months on board Mir

Survival

Eight adult animals were launched in space, but only two females stayed alive for five months on board Mir. The 17th day after the launch, the four males died in the CTA. Two females were transferred to the instrument "Fertile" and the two others were kept in the CTA. On the 18th day, the two females in the instrument "Fertile" died. The two surviving females were kept in the CTA, but with only one female per rearing box. They were alive at landing. In the synchronous ground experiment, 4/4 females and 3/4 males were alive at the end of the space mission. One male having died three weeks after the beginning of the ground control experiment.

Feeding

Before and after the space experiment, all the experimental and control animals were weighed (Table 3). Batches of 2 in-flight and 7 control animals were force-fed during 5 months onboard Mir and in the ground laboratory, respectively. The in-flight females (N = 2) lost 7 g of weight on average (-16 %). In the control batch, the females (N = 4) and the males (N = 3) lost 2.5 g of weight on average (-6 %) and 6 g of weight (-25 %), respectively.

Physiological status of the animals

Between two months and two weeks before the launch of the space mission, four experimental and four control females were inseminated by a natural mating and laid fertilized eggs. The females keep spermatozoa in their cloacal glands alive after a natural mating and can lay fertilized eggs again in absence of male if they are hormonally stimulated. During the mission, the two surviving in-flight females and two ground control females received an injection of LH-RH to induce ovulation and laying of fertilized eggs that developed into embryos and larvae *(Aimar et al., 2000)*. For these females, this second laying occurred on board Mir 27 days after the launch for one female and 36 days after the launch for the other female. During the first week following the landing, two in-flight and the four control

Table 3. Weight of in flight and synchronous ground control animals force-fed during 5 months.

	Number	Weight at the space mission (g)	Weight after the five-month space mission (g)	
In flight females	2	45.0 ± 2.0	38.0 ± 0.5	s.
Ground females	4	45.0 ± 1.8	42.5 ± 2.7	n.s.
Ground males	3	24.3 ± 1.2	18.3 ± 2.0	s.

females were mated with standard males. They laid fertilized eggs, except for one ground-control female.

Discussion

The force-feeding technique has been commonly used in mammals but seldom employed in amphibians *(Mather and Ahmad, 1974; El-Mofty and Sakr, 1988; Lessire, 1990; El-Mofty, Khudoley and Shwaireb, 1991; Salvado and Arola, 1994)*. Such technique was never previously performed on an amphibian in space conditions, although treefrogs have been kept on board Mir and fed with worms for 8 days *(Yamashita et al. 1997)*. The adult amphibians previously used in space experiments did not stay more than three weeks in space and did not need food during that time *(Souza et al., 1995; Mitashov et al., 1996; Yamashita et al. 2001)*.

In our previous space experiment performed two times on board Mir, 12 adult females stayed on board the station in the same instrument "Fertile" used for the Genesis experiment. These preinseminated females fasted, laid eggs in flight, and came back alive after 16- and 21-day journeys in space *(Dournon et al., 2001; Gualandris-Parisot et al., 2002)*. Therefore, as females could fast for three weeks, poor feeding did not cause the loss of the four males and two females at the beginning of the 1999 space mission Perseus. In the absence of any identified fault in the CTA or the "Fertile" instrument, the death of these animals had no clear explanation. Since the CTA was a new instrument and the instrument "Fertile" had been 2.5 years under space conditions, we decided to use the CTA which had a recently serviced air pump. A few months after the space mission, we learnt that a breakdown in the ventilator system of the space station occurred during the corresponding period in the area where the CTA and the instrument "Fertile" were located. Consequently, in absence of convection, a bubble of gas produced by the respiration of a cosmonaut, which slept in this area, might have enveloped the instruments and remained in their vicinity. So, the animals were consequently ventilated with air containing a reduced O_2 concentration. The morphological aspect of the dead animals observed in videomovies was in accordance with anoxia. Immediately after the death of males, we decided to open and ventilate one time a day the two boxes containing two females each. However, this procedure was not efficient, as two females died in the same box. After this event, the two remaining females were placed in separate boxes, which were ventilated twice a day until the end of the space mission i.e. during four months. As a result, the two females were alive at landing. On the ground, seven among eight control animals were alive at the end of the experiment.

During the 5-month space experiment, the loss of weight 4 and 10 g, respectively of the two space females force-fed three times a week was significant (Table 3). Nevertheless, these females layed eggs during the space mission. On the ground, we have observed that when females lay eggs, the total weight of eggs (more than 800 eggs) and of the dehydrated jelly coat was about 4 g (8-9 % of body weight). After the landing, the space females were in good condition, and after mating with males, they again layed fertilized eggs. Concerning the weight of the synchronous ground control animals, the difference was not significant for the females but was for the males (Table 3). These females were also in good condition and layed fertilized eggs, but the males were thin and were not mated.

According to the results obtained with *Pleurodeles* females, the diluted preparation could be used and improved in future long space flights. However the results with the ground control males that could not reproduce indicated that the food must be improved. As a matter of fact, the weight loss could be caused by a regurgitation phenomenon and consequently by a too small quantity of food received by the males. The standard males are thin and the 2.1 mm in diameter catheter takes up the volume of the esophagus and a part of the stomach. Therefore, the nutritive quality of the food, must be increased. The two space females and the ground control ones were used to analyze 1) the fertilization and further development of embryos issued from oocytes in which a vitellogenesis part occurred in microgravity conditions, 2) the morphological aspect, crystallographic structure and chemical composition of otoconia taken from the inner ear, 3) the immunoglobin expression. The analysis of these experiments is in progress.

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